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1 **A generic coordination assembly-enabled nanocoating of individual tumor cells for**
2 **personalized immunotherapy**

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21
22 Keywords: Cell encapsulation, microparticles vaccine, tumor immunotherapy, metal–organic
23 coordination, murine melanoma

24 A generic and effective tumor cells encapsulation strategy enabled by metal–organic
25 coordination is developed to prepare vaccine for personalized immunotherapy. Specifically,
26 epigallocatechin-3-gallate (EGCG)-Al(III) coordination layer is *in situ* formed onto individual
27 living cell in aqueous phase, and the process can be completed within an hour. 98% of proteins
28 in cells are entrapped within the microparticles, which endow with high antigens loading
29 capacity. The microparticles enhance the uptake efficiency of antigens, protecte antigens from
30 degradation in vivo, and delay the retention time of antigens in lymph node. Moreover, dendritic
31 cells (DCs) activation is triggered by the microparticles, simultaneously, the expression of co-
32 stimulation marker on DCs and the production of Th1-related cytokines are significantly up-
33 regulated. Moreover, six kinds of tumor cells are utilized and successfully coated by
34 EGCG/Al(III) layer, suggesting the generalization of this strategy. More importantly, the
35 microparticles exhibit the comparative antitumor effect with polyinosinic-polycytidylic acid

1 (PolyI:C) in B16 pulmonary metastasis model. Overall, the encapsulation strategy enabled by
2 metal–organic coordination could be potentially useful for personalized immunotherapy
3 customized to individual patient’s tumor cell.

4 **1. Introduction**

5 Immunotherapy that regulates the body’s immune system to resist diseases, is one of the most
6 promising therapeutic strategies for metastatic cancer.^[1] The main types of immunotherapy
7 include tumor vaccines, cell transfer therapy (DCs, NK, and T cells), and immune checkpoint
8 blockades (CTLA-4, PD-1 and PD-L1).^[2] Of which, tumor vaccines consist of defined antigens,
9 aiming to treat and prevent tumor occurrence or recurrence after surgery. However, the
10 development of tumor vaccines remains a challenge due to the lack of antigen, the low uptake
11 efficiency and poor immunogenicity of antigen.^[3]

12 Whole tumor cell lysates offer a rich source of antigens and can serve as multivalent
13 vaccines against tumor.^[4] There are four main ways to prepare tumor lysates: freeze-thaw cycles,
14 UVB irradiation/irradiation, hypochlorous acid-oxidation, and hyperthermia.^[5] Before the
15 formation of tumor lysates, tumor cell can be modified genetically to secrete pro-inflammatory
16 cytokines, inhibit immunosuppressive cytokines or overexpression of calreticulin.^[6] To further
17 improve the efficiency, tumor lysates can be used in association with adjuvants (IL-2) or
18 immunomodulator (CpG, MPLA and PolyI:C),^[7] or to pulse DCs for ex vivo DC-based
19 therapy.^[8] Recently, biodegradable particles (50 nm~10 μm) have been engineered to
20 effectively deliver tumor lysates and activate DCs, including CaCO₃ microparticles,^[9] chitosan
21 nanoparticles,^[10] yeast cell wall particles,^[11] and PLGA particles.^[12] Moreover, tumor lysates-
22 derived membrane proteins were engineered into nanoliposomes,^[13] PLGA nanoparticles.^[14]
23 David Mooney and colleagues co-encapsulated tumor lysates, GM-CSF and CpG into
24 injectable cryogel for DCs recruitment and immunotherapy.^[15] Besides, irradiated tumor cells
25 coated with immunosuppressive blocker/immunocytokine were prepared as whole tumor cells
26 vaccines.^[16] Various strategies have been exploited to make whole tumor cell vaccines, for

1 example, tumor vaccine was obtained after knockdown the inhibitor of differentiation protein
2 2 in whole tumor cells.^[17] However, the direct encapsulation of individual living tumor cells as
3 tumor vaccines is rarely reported. Except in one report^[18] using LbL assembly of poly(N-
4 vinylpyrrolidone) and tannic acid onto the surface of tumor cells, yet DCs activation and the in
5 vivo efficacy of the vaccine was not tested.

6 Epigallocatechin-3-gallate (EGCG), the major polyphenol in green tea, has demonstrated
7 beneficial functions including anti-cancer, anti-HIV, anti-inflammatory effects, etc.^[19] EGCG
8 can modulate macrophages functions by upregulating Th1 cytokines (IL-12, TNF- α and INF-
9 γ), and downregulating immunotolerance-related cytokines (IL-10).^[20] Furthermore, EGCG can
10 recover T cell suppression via inhibiting the IDO expression on tumor-associated antigen
11 presentation cells (APCs).^[21] Therefore, the use of EGCG as a vaccine component may be able
12 to break the tolerance of tumor immunotherapy. Al(III) is the main component of aluminum
13 adjuvant, which is the most commonly used adjuvant in humans with the capacity to provoke a
14 strong humoral immunity. It was reported that EGCG can coordinate with Al(III) to form
15 soluble EGCG-Al(III) complexes.^[22] Moreover, Caruso and colleagues found that the
16 coordination of tannic acid and Fe(III) can be deposited onto particle templates (such as SiO₂,
17 CaCO₃ and PS spheres),^[23] and yeast cells.^[24] Inspired by these previous studies, we speculate
18 that the coordination layer of EGCG-Al(III) can be formed onto the tumor cells, which provided
19 a novel strategy for whole tumor cell vaccines that integrate immunostimulatory agents in well-
20 defined nanocoatings of single tumor cells.

21 In this study, a generic strategy to prepare tumor microparticle vaccines were developed by
22 nanocoating of individual living tumor cells with EGCG/Al(III) coordination layer. In our
23 previous work,^[25] the strategy to encapsulate individual B16 tumor cells was verified;
24 meanwhile, the uptake of microparticles vaccine by DCs, and their therapeutic effect in B16
25 subcutaneous tumor model were also investigated. This work focused on the universality of this
26 strategy to other tumor cells and the interaction between the microparticles vaccine and antigen

1 presentation cells (APCs) including their cytotoxicity to DCs and RAWs, and DCs activation
2 and maturation. Furthermore, *in vivo* trafficking of the microparticles vaccines and the anti-
3 metastatic effect of this microparticles vaccine in B16 pulmonary metastases model were also
4 investigated. This strategy could retain almost all of the antigens and avoid time-consuming
5 and costly antigen epitope identification. Five kinds of mouse tumor cells and one kind of
6 human tumor cells were utilized to prove the universality of this strategy. The coordination
7 assembly-enabled tumor cells encapsulation strategy has the clinical potential of personalized
8 immunotherapy.

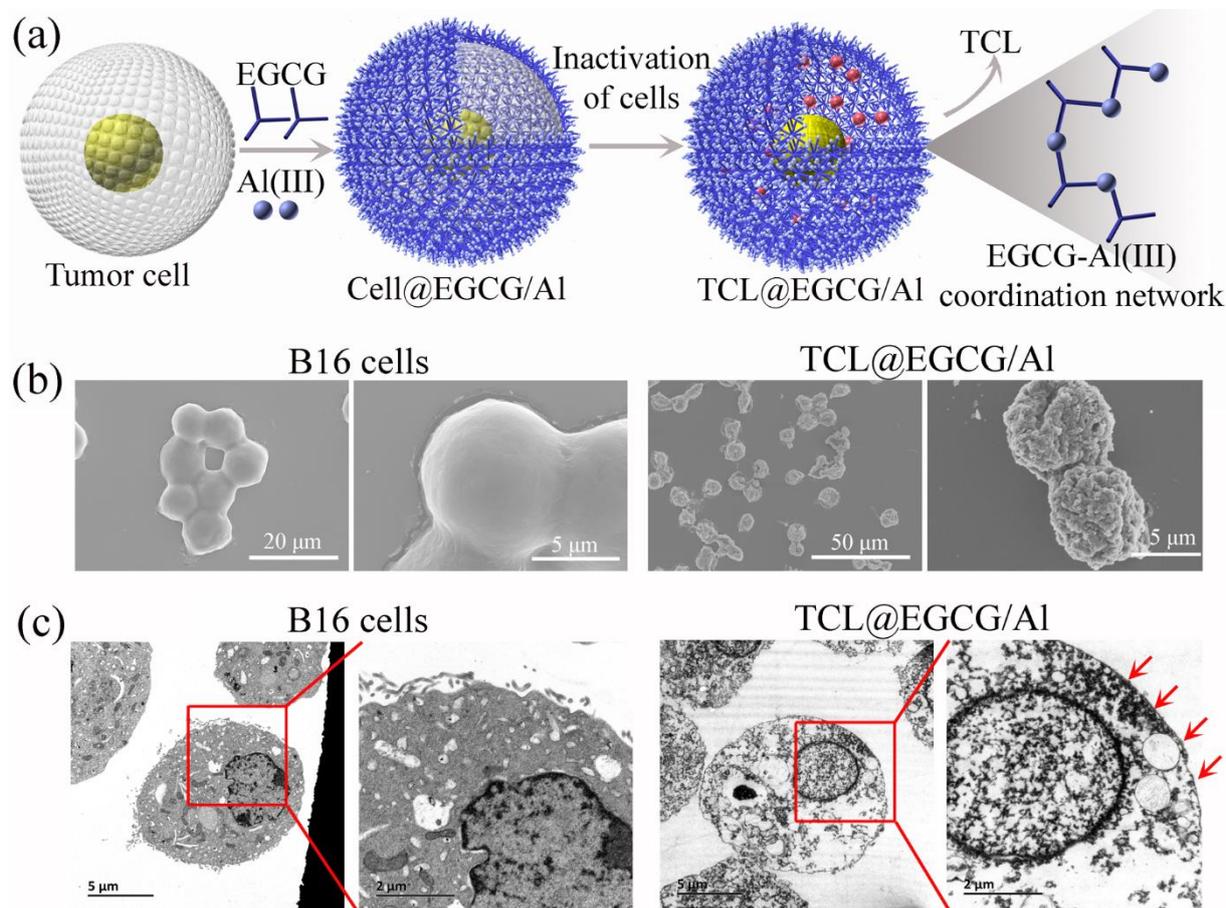
9 **2. Results and Discussions**

10 **2.1. Encapsulation of tumor cells and preparation of TCL@EGCG/Al**

11 A generic approach to coat individual living tumor cells with EGCG/Al coordination layers
12 has been developed for the construction of tumor microparticles vaccine. The vaccine could be
13 potentially useful for personalized immunotherapy customized to individual patient's tumor
14 cells. **Figure 1a** illustrated the preparation approach. Tumor cells were coated with EGCG/Al
15 layers by suspending in EGCG and $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ aqueous solutions for 60 s. The process was
16 repeated three times, leading to the formation of EGCG/Al₃ shell, respectively. After
17 inactivation of the cells upon hypotonic treatment, the microparticles vaccine contained tumor
18 cell lysate (TCL@EGCG/Al) were obtained.

19 The surface of the microparticles was rough and the size appeared homogeneous with an
20 average diameter of 7 μm , smaller than that of live B16 cells due to the evaporation of water
21 (Figure 1b). In comparison with TCL@EGCG/Al, uncoated B16 cells had a smoother surface.
22 TEM images of ultrathin sections were further used to investigate the internal structure of
23 TCL@EGCG/Al microparticles. As depicted in Figure 1c, a continuous layer encompassed the
24 cell membrane, cytoplasm and cell nucleus, confirming the successful encapsulation of
25 individual tumor cells with EGCG/Al layer. Besides, abnormal cell structures together with

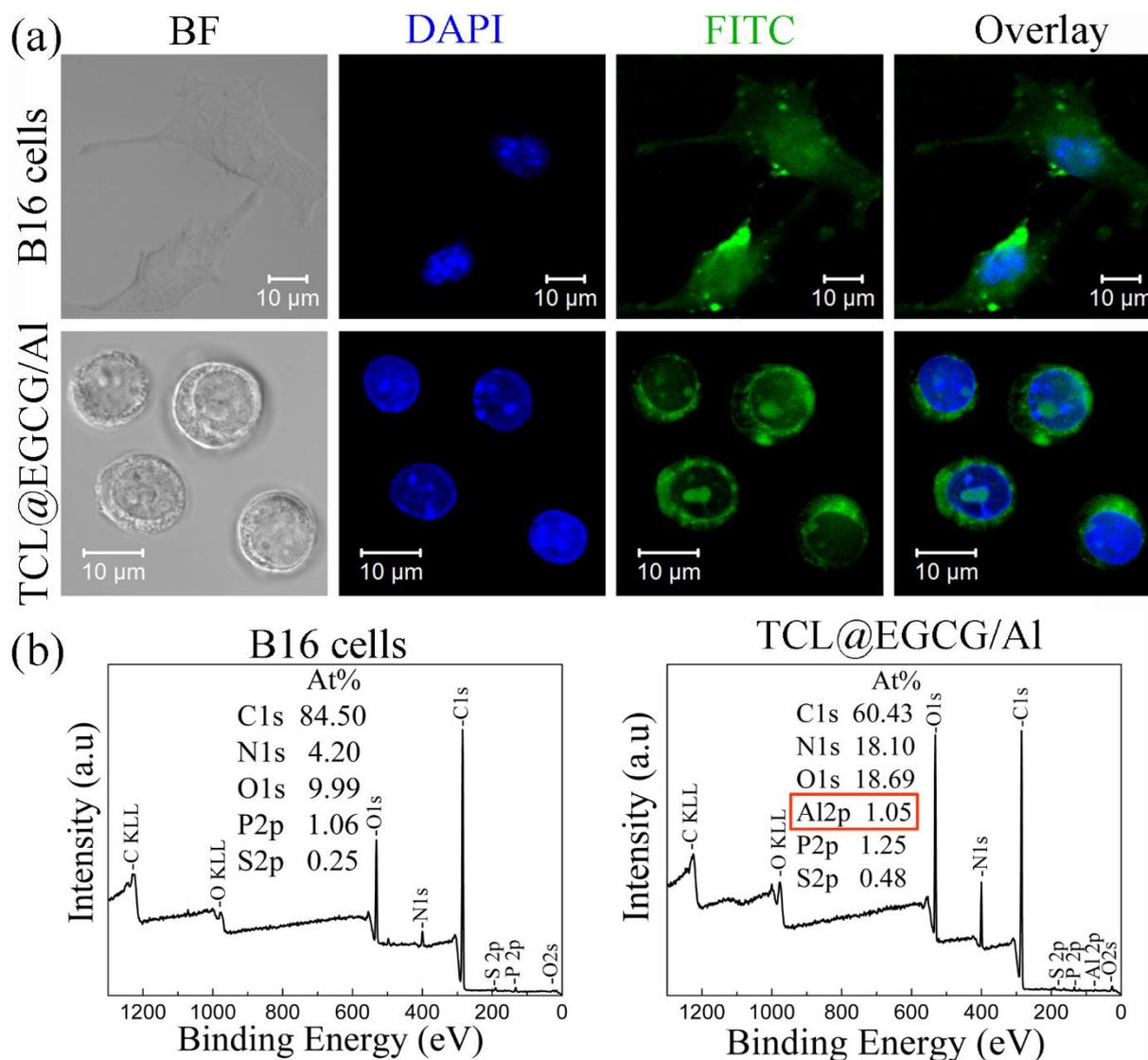
1 degeneration of the cytoplasm and the cell nucleus were observed in TCL@EGCG/Al,
 2 suggesting the inactivation of tumor cells and excluding the oncogenicity of the microparticles
 3 vaccines.



4
 5 **Figure 1.** (a) Preparation of TCL@EGCG/Al microparticles vaccine, SEM (b) and TEM (c)
 6 images of B16 cells and TCL@EGCG/Al. The red rectangles showed a zoomed area, and the
 7 red arrows indicated the EGCG/Al coordination layer.

8 From CLSM images (**Figure 2a**), significantly different morphology of TCL@EGCG/Al
 9 was observed in comparison with live B16 cells, further suggesting the successful coating of
 10 cell. Besides, the size of TCL@EGCG/Al was $\sim 13 \mu\text{m}$, which was comparable with the size of
 11 B16 cells. Besides, dsDNA stained by DAPI was observed, indicating that the preparation
 12 process had little effect on the structure of genomic DNA. Moreover, X-ray photoelectron
 13 spectroscopy (XPS) spectrum of TCL@EGCG/Al indicated the presence of C, N, O, P, S and
 14 Al elements (Figure 2b). N, P, S and some C, O elements originated from the cells. Al and some

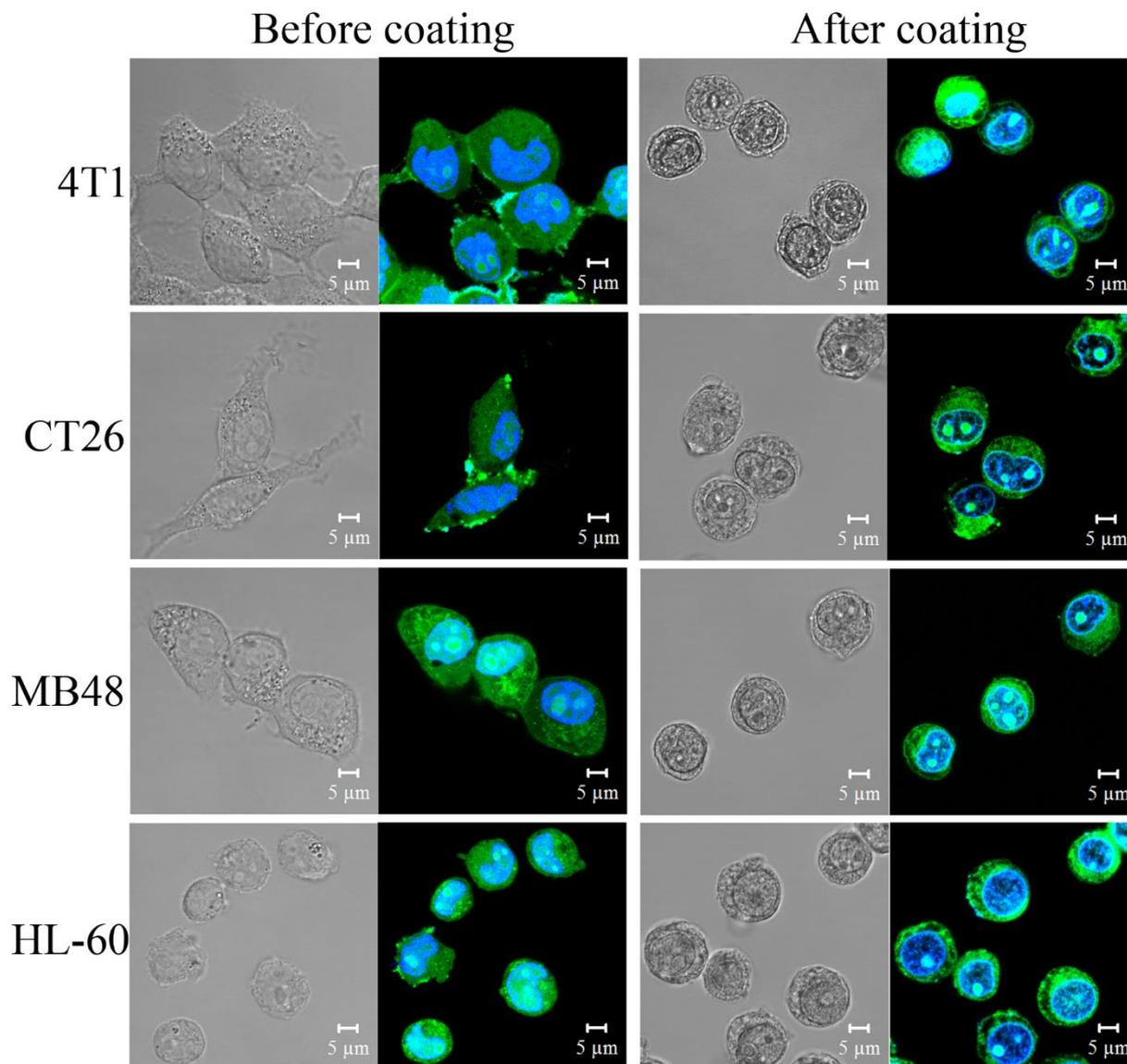
1 C, O elements came from the EGCG/Al layer. In contrast, Al element was not observed in
 2 uncoated B16 cells, indicating the formation of EGCG-Al coordination layer onto cells surface.



3
 4 **Figure 2.** CLSM images (a) and XPS spectrum (b) of B16 cells and TCL@EGCG/Al.

5 The EGCG-Al(III) coordination layers formed rapidly independent of the nature of substrate,
 6 suggesting that the approach can be extended to other cell types. B16 cells were replaced by
 7 mouse MB49, CT26 and 4T1 cells line, and human HL-60 cells line. CLSM images showed
 8 that the distinct changes in cell morphology and intracellular structure after coating tumor cells
 9 (**Figure 3**). SEM images (**Figure S3**) showed that the size of microparticles was consistent with
 10 the cell size, but had a quite different surface topography with these templated cells. Compared
 11 to uncoated cells, the coated cells had a rougher surface. CLSM and SEM images illustrated

1 that the microparticles were successfully prepared with the four types of tumor cells using the
2 identical procedure. These results demonstrate the universality of this method, which could be
3 applied to prepare other cells-related formulations.



4
5 **Figure 3.** CLSM images of 4T1, CT26, MB49 and HL-60 cells before and after coating.

6 During the process, cells were repeatedly incubated in EGCG and AlCl_3 aqueous solution
7 followed by centrifuging/washing. CCK-8 assay was used to investigate the effect of these
8 mechanical procedure on cell viability, PBS control underwent the same mechanical procedure
9 as TCL@EGCG/Al with addition of EGCG and AlCl_3 solution. As displayed in **Figure 4a**, the
10 coating of EGCG/Al layer displayed significant influence on cell viability regardless of B16,

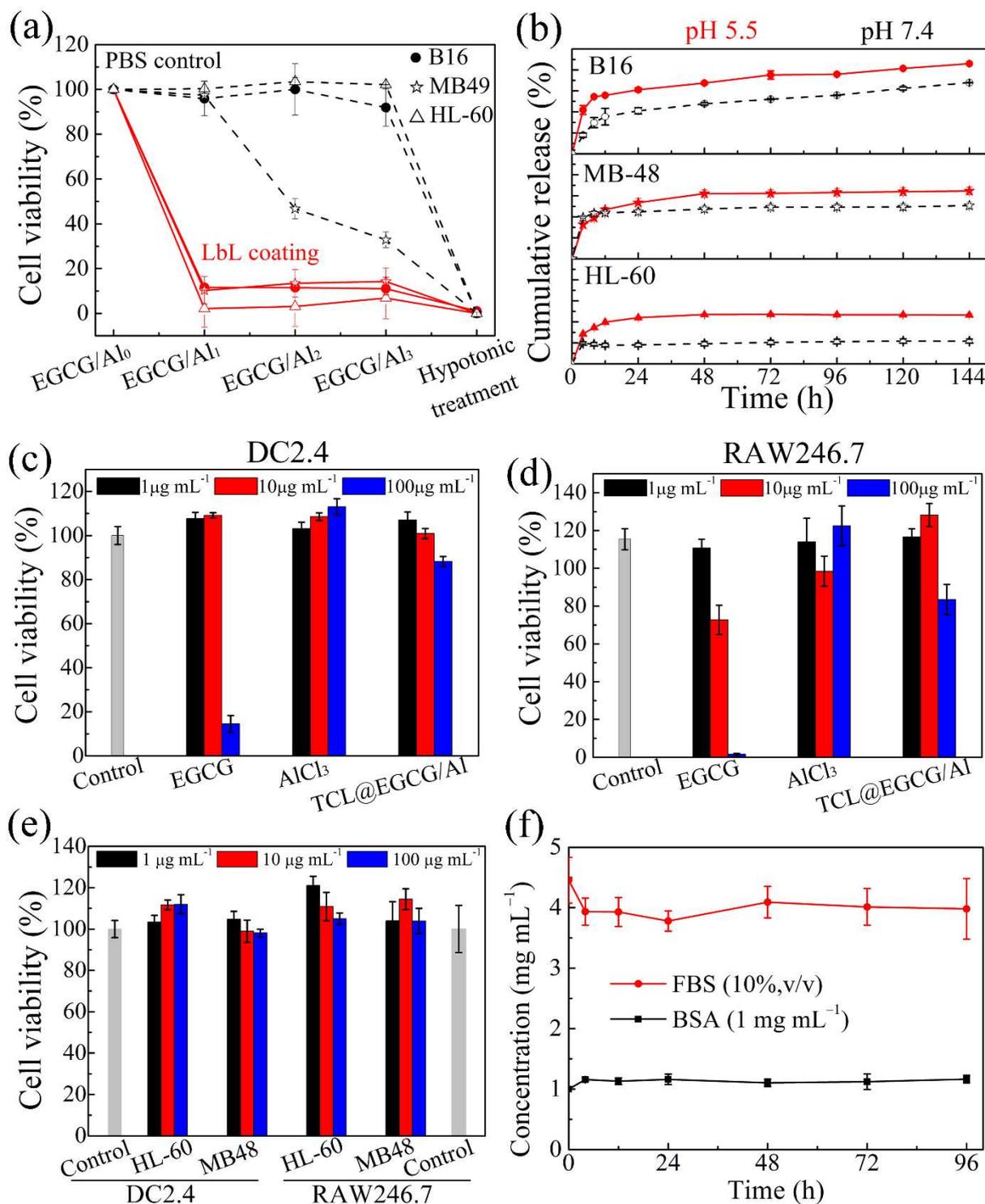
1 MB48 or HL-60 cells, suggesting that the effect of EGCG/Al coating on cell viability were the
2 same. As for PBS control, the cell viabilities of B16 and HL-60 cells were not affected by these
3 mechanical procedures; while these mechanical procedures displayed significant influence on
4 cell viability of MB48. To ensure safe use and avoid the oncogenicity, the coated cells were
5 further exposed to hypotonic medium by incubating them with high-purity deionized water.
6 Upon exposure to hypotonic medium, cellular proteins might diffuse through the EGCG/Al
7 layer, leading to the loss of tumor antigen. Measured by BCA assay, the protein amount of B16
8 cells was 1004.7 ± 14.9 pg/cell. The protein amount and loading capacity of TCL@EGCG/Al
9 prepared from B16 cells were 984.5 ± 4.9 pg/microparticle and 497.7 ± 2.5 $\mu\text{g mg}^{-1}$ vaccine,
10 respectively. About 98% of proteins in tumor cells was entrapped within TCL@EGCG/Al,
11 avoiding the loss of tumor antigens. The protein loading capacity is higher than those of other
12 vaccine delivery carriers such as PLGA NPs,^[26] polyelectrolyte coated AuNPs,^[27] and
13 mesoporous silica NPs.^[28]

14 The protein release rate of microparticles was pH-dependent (Figure 4b). TCL@EGCG/Al
15 prepared from B16 cells exhibited fast protein release at pH 5.5, with 60-70% of protein
16 released within the initial 24 h. At pH 7.4, only 30-40% of the total protein was released within
17 24 h. This can be ascribed to that the formation of coordination bonds between EGCG and Al^{III}
18 is pH dependent.^[29] The pH-dependent protein release was attributed to the decreased stability
19 of EGCG-Al(III) complexes with decreasing pH.^[30] The difference in stability at extracellular
20 physiological pH (7.4) and intracellular endo/lysosome pH (5.5) was favorable for the enhanced
21 intracellular release of protein antigens. TCL@EGCG/Al prepared from MB48 or HL-60 cells
22 exhibited similar protein release profile with that prepared from B16 cells.

23 DCs, the most potent APCs, are necessary for inducing protective immunity, and
24 macrophages are another key type of APCs. Therefore, the biocompatibility of
25 TCL@EGCG/Al prepared from B16 cells and their respective individual constituents on DC2.4
26 and RAW264.7 cells were assessed (Figure 4c-d). Although EGCG exhibited distinct

1 cytotoxicity to DC2.4 and RAW246.7 cells at test concentrations of 100 $\mu\text{g}/\text{ml}$ due to its strong
2 reducibility. TCL@EGCG/Al and AlCl_3 had no distinct cytotoxicity at concentrations below
3 100 $\mu\text{g mL}^{-1}$, with more than 80% of cell viability after incubation with DC2.4 and RAW246.7
4 cells for 48 h. In addition, TCL@EGCG/Al prepared from HL-60 or MB48 cells has relatively
5 negligible cytotoxicity to DC2.4 and RAW246.7 cells, with more than 100% of cell viability
6 after incubation for 48 h (Figure 4e). Consequently, the cytotoxicity of the microparticles
7 vaccine prepared from different tumor cells on APCs can be negligible.

8 Furthermore, the stability of TCL@EGCG/Al was also evaluated by suspending the
9 microparticles vaccine in culture medium containing a known concentration of BSA or FBS
10 and incubated at 37 °C. As shown in Figure 4f, the concentration of BSA in the suspension
11 retained almost constant over the period of incubation, suggesting that the microparticles
12 vaccine had a high stability in 1 mg mL^{-1} of BSA solution. Then, a higher protein concentration
13 of FBS (10 v/v%, 4.46 mg mL^{-1}) was used to incubate with the microparticles vaccine. Only
14 about 10% of FBS was adsorbed on the microparticles vaccine over the period of incubation
15 (Figure 4f). These results revealed that the microparticles vaccine was not prone to fouling by
16 proteins in the extracellular environment. Besides, optical images were taken after incubation
17 for different time (**Figure S4**). Monodisperse microparticles vaccine was observed. Neither
18 aggregation of microparticles or protein-aggregated microparticles was formed.

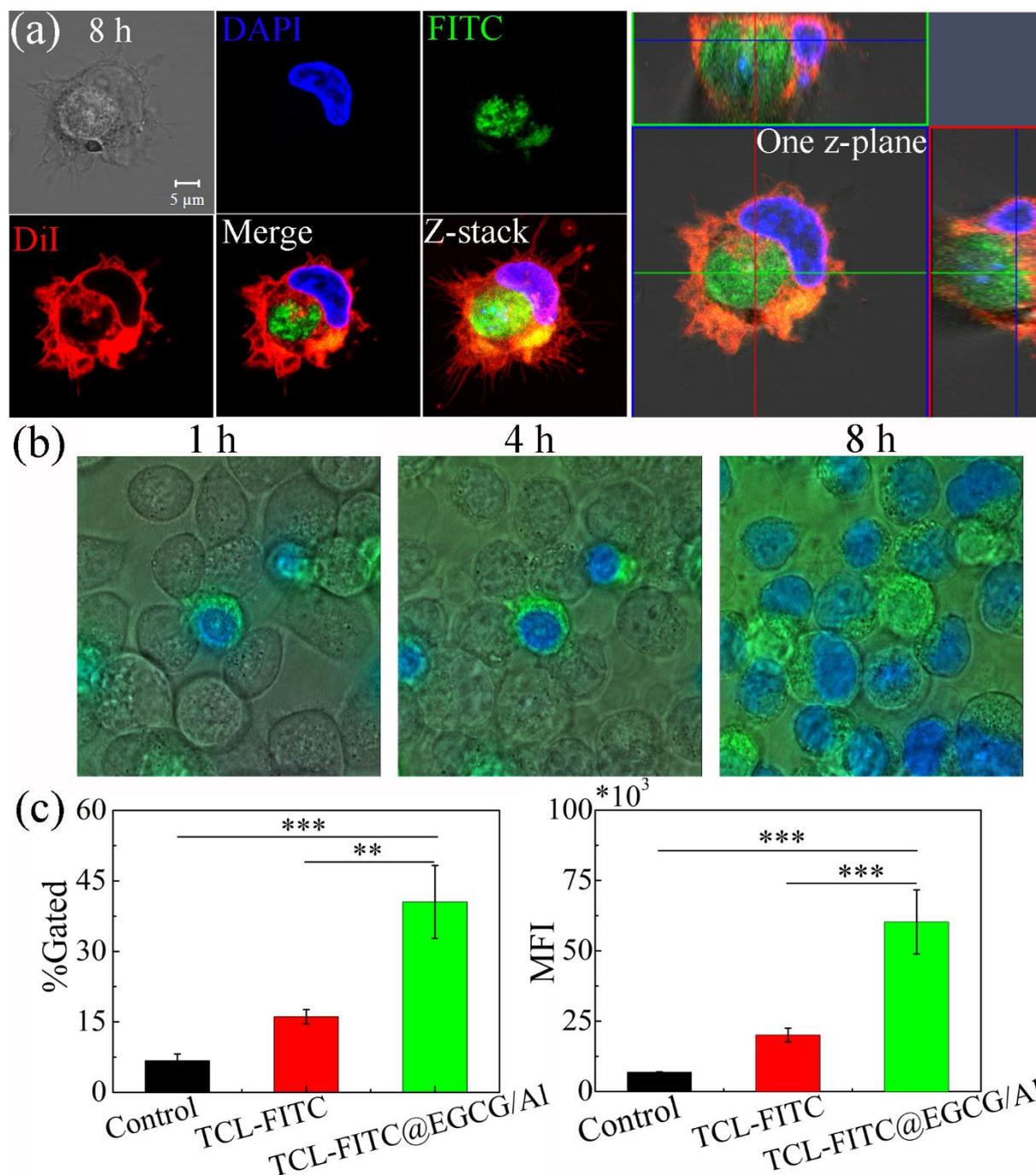


1
2 **Figure 4.** (a) Cell viability of B16, MB48 and HL-60 cells after each deposition cycle during
3 preparation of TCL@EGCG/Al, the data represent mean \pm SD ($n = 3$) (b) Time-dependent
4 release of proteins from TCL@EGCG/Al prepared with B16, MB48 and HL-60 cells at pH 5.5
5 and 7.4, Cell viability of DC2.4 (c) and RAW246.7 (d) cells after incubating with
6 TCL@EGCG/Al prepared with B16 cells and the respective materials for 48 h, (e) Cell viability

1 of DC2.4 and RAW246.7 after incubating with TCL@EGCG/Al prepared with MB48 and HL-
2 60 for 48 h, culture medium was used as control, the data represent mean \pm SD (n = 6), (f) The
3 concentration of BSA and FBS as a function of time, the initial concentration of BSA and FBS
4 (10%, v/v) was 1 mg mL⁻¹ and 4.46 mg mL⁻¹, respectively, the data represent mean \pm SD (n =
5 3).

6 **2.2. Antigens uptake and BMDCs activation *in vitro***

7 BMDCs were pulsed with TCL-FITC@EGCG/Al and then observed by CLSM. From CLSM
8 images (**Figure 5a**), the entire microparticles could be phagocytized by BMDCs although their
9 size was quite larger. After endocytosis of the microparticles, BMDCs have prominent dendritic
10 structures from the Z-stack CLSM image, suggesting their maturation. Live cell imaging
11 systems were used to further reveal the endocytosis of microparticles vaccine. Considering that
12 BMDCs is a mixed population of macrophages and DCs, its purity is based on the expression
13 levels of CD11c. Accordingly DC2.4 is more appropriate for real-time observation of the
14 microparticles endocytosis by live cell imaging systems, because it provided more pure
15 population of DCs. The result demonstrated that the antigens entrapped in the microparticles
16 vaccine can also be phagocytized by DC2.4 cells (Figure 5b and Supporting Information Video
17 S2). It can be seen that both the entire microparticles and their debris can be phagocytized by
18 DCs. Furthermore, FACS was further used to quantify antigen uptake (Figure 5c). About 2.5-
19 fold increase of positive cells and 3-fold increase of the mean fluorescence intensity (MFI) were
20 observed after co-culturing with TCL-FITC@EGCG/Al as compared to soluble TCL-FITC.
21 This result suggested that the microparticles can significantly increase antigen uptake by DCs
22 compared to soluble antigens.

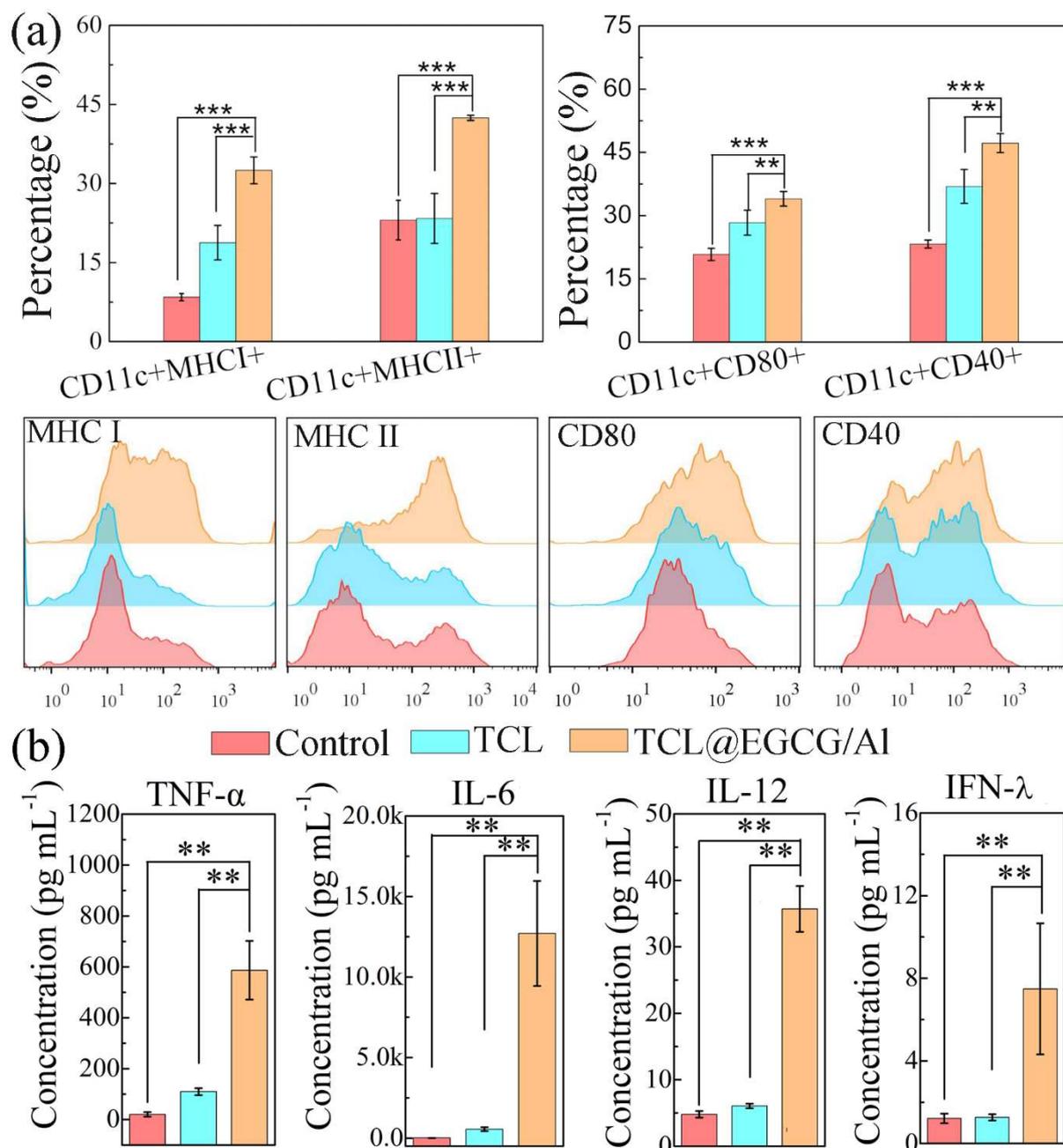


1
 2 **Figure 5.** (a) CLSM images of BMDCs pulsed with TCL-FITC@EGCG/Al for 8h, showed the
 3 maximum intensity projection of the full z-stack and one z-plane plus corresponding cross
 4 sections, (b) Optical images of DC2.4 incubating with TCL@EGCG/Al obtained by live cell
 5 imaging systems, (c) DC2.4 cells were incubated with TCL-FITC and TCL-FITC@EGCG/Al
 6 for 6 h, the percentage of positive cells (%Gated) and the MFI detected by FACS, the data are

1 expressed as mean \pm SD (n =3), and the differences were analyzed by one way ANOVA with
2 Bonferroni multiple comparison post-test, **p < 0.01, ***p < 0.001.

3 After antigen uptake, DCs processed and presented antigens to T cells. During the process,
4 DCs were matured and expressed activation markers to prime T cells. BMDCs maturation was
5 characterized by surface marker expression using FACS analysis (**Figure 6a**). Untreated cells
6 served as control. TCL@EGCG/Al induced up-regulation of CD40 and CD80 on BMDCs
7 surface in comparison to the untreated cells and soluble TCL. Furthermore, the expression
8 levels of MHC I and MHC II were also significantly up-regulated, indicating a facilitative role
9 of TCL@EGCG/Al on BMDCs maturation and an enhanced capability to initiate CD8+ and
10 CD4+ T cell priming via MHC I and MHC II pathway, respectively. These observations
11 demonstrated the ability of TCL@EGCG/Al to induce BMDCs maturation and activation, and
12 enhance the antigen presentation ability of BMDCs.

13 The expression levels of different inflammatory mediators released in the culture medium
14 were also investigated. In comparison with soluble TCL, TCL@EGCG/Al strongly enhanced
15 expression levels of Th1-related cytokines IL-12p70, TNF- α , IL-6 and IFN- γ (Figure 6b), but
16 not of Th2-inducing cytokine IL-4 and IL-10 (**Figure S5**). Specifically, TCL@EGCG/Al
17 caused more than 5-fold increase in the level of cytokines (IL-12p70, TNF- α , and IFN- γ) and
18 23-fold increase in the level of IL-6 compared to soluble TCL. IL-6 is the recruiting cytokines,
19 play a surprisingly crucial role in APCs recruitment in vivo.^[31] Therefore, APCs can be
20 recruited to the vaccine injection site under the regulation of IL-6. TNF- α was a characteristic
21 proinflammatory cytokine with anticancer activity, played a key role in destroying the tumor
22 vascular endothelia. Mature DCs and the IL-12 they produce allow T cells to differentiate into
23 IFN- γ -producing Th1 cells.^[32] IFN- γ together with IL-12 promote the differentiation of T cells
24 into CD8+ CTL cells. Therefore, the microparticles triggered the maturation of DCs and the
25 production of Th1-related cytokines revealed their great antitumor potential.



2 **Figure 6.** (a) The expression levels of MHC I, MHC II, CD80 and CD40 on CD11c⁺ BMDCs
 3 determined by FACS and their representative FACS histograms, the differences were analyzed
 4 using one-way ANOVA with Bonferroni multiple comparison post-test, (b) Secretion of TNF-
 5 α , IL-6, IL-12P70 and IFN- γ from BMDCs treated with different formulations, the differences
 6 were analyzed using unpaired student's t-test. Data are expressed as mean \pm SD (n = 3), **p <
 7 0.01, ***p < 0.001.

1 2.3. *In vivo* trafficking of TCL@EGCG/Al

2 Upon antigen uptake and DCs activation, DCs have to reach the lymph nodes, where they
3 will encounter naïve T cells and initiate a specific immune response. The migration process was
4 monitored in real-time *in vivo*. **Figure 7a** shows the representative images of fluorescence
5 signals corresponding to TCL-Cy7 and TCL-Cy7@EGCG/Al at designated time points. The
6 average fluorescence in dLN and injection site were quantified and plotted against time in
7 Figure 7b. After injection for 6 h, Cy7 signal was recorded in dLN for both TCL-Cy7 and TCL-
8 Cy7@EGCG/Al groups, suggesting that the antigen has been migrated to dLN. After injection
9 for 48 h, no signal of Cy7 was recorded in LN for TCL-Cy7 group whereas robust and sustained
10 Cy7 signal was detectable in of TCL-Cy7@EGCG/Al group. This result indicated that the as-
11 prepared microparticles can effectively protect antigens against degradation *in vivo* and delayed
12 the retention time of antigens in lymph node. After injection, the mean density of Cy7 for TCL-
13 Cy7 retained a constant value in the injection site within 48 h. While the mean density of Cy7
14 for TCL-Cy7@EGCG/Al was distinctly low after 0 hours of injection due to aggregation-
15 caused quenching (ACQ).^[33] After 6 hours of injection, the mean density of Cy7 was increased
16 and afterward retained a constant value, suggesting that TCL-Cy7 have been uptaken by APCs,
17 thus ACQ phenomenon was eliminated.

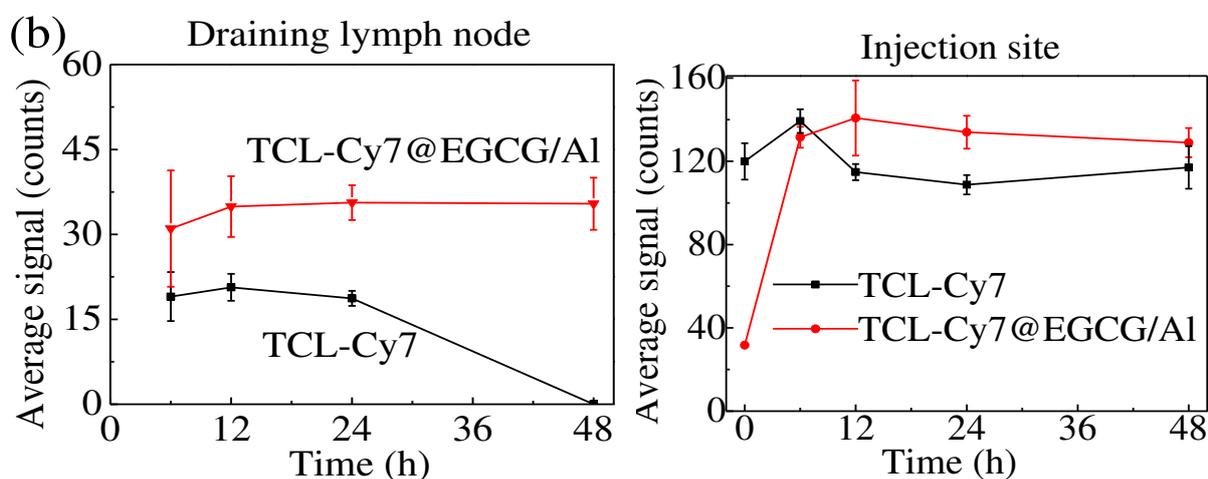
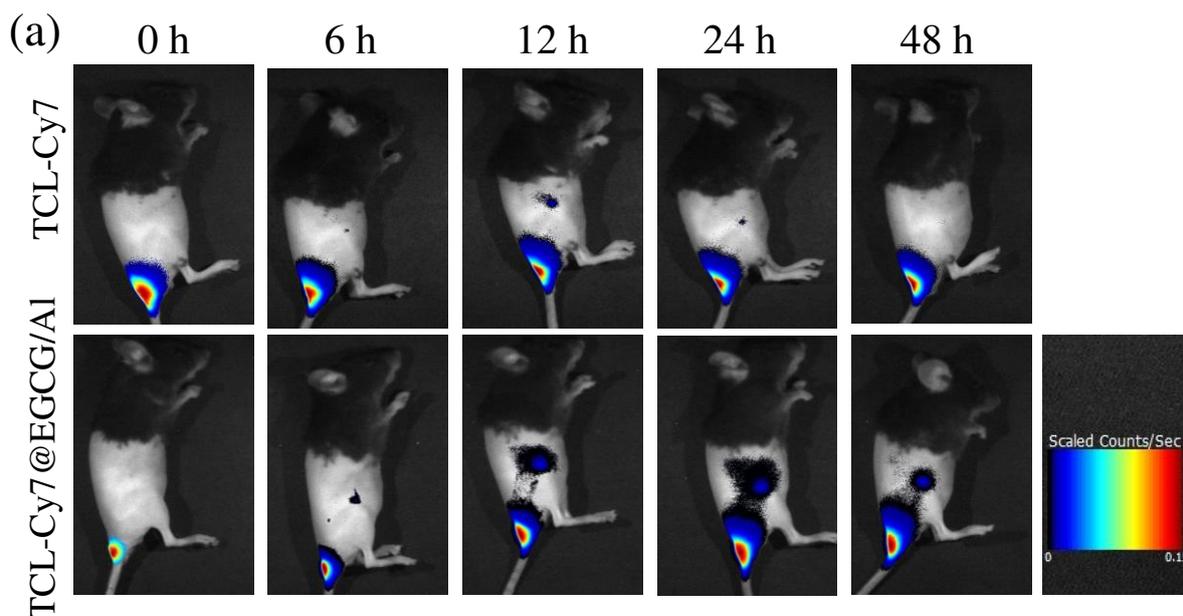


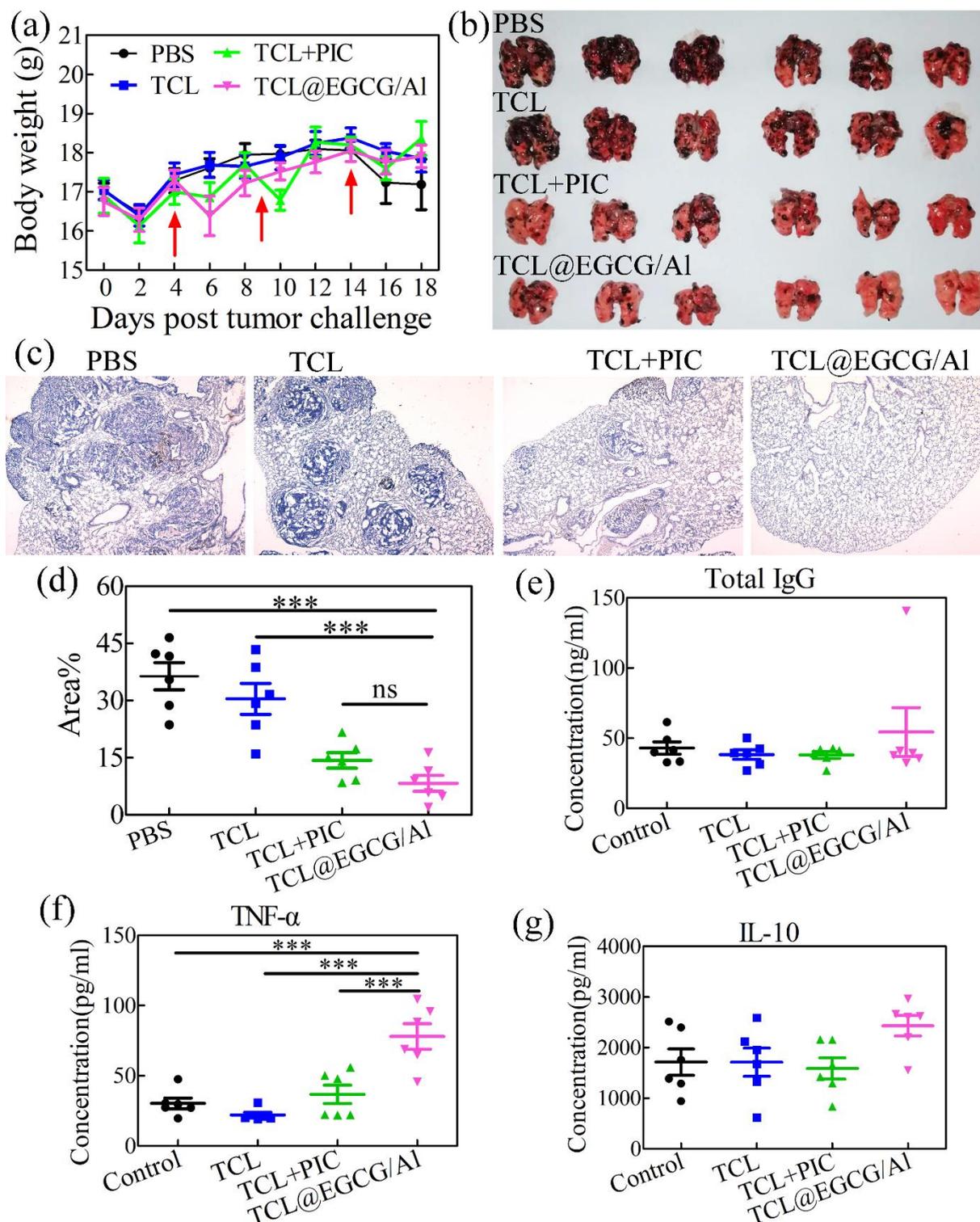
Figure 7. (a) Presence of TCL-Cy7 and TCL-Cy7@EGCG/Al in the injection site and the right draining lymph node, all images were overlays of bright photographs with fluorescence intensity measurement. (b) The mean density of Cy7 in the draining lymph node and the injection site. Data are expressed as mean \pm SD (n =3).

2.4. Antitumour immunity of TCL@EGCG/Al in B16 pulmonary metastasis

In order to validate the antitumor efficacy of TCL@EGCG/Al in vivo, B16 pulmonary metastases model was established. PolyI:C (abbreviated as PIC) as TLR-3 agonist has been evaluated as a vaccine adjuvant to enhance the specific antitumor immune responses.^[34] Therefore, PIC+free TCL was used as the positive control. On day 4 post inoculation of B16 cells, mice were subcutaneously immunized with various formulations for three times

1 accordingly. The vaccinations including TCL, TCL+PIC and TCL@EGCG/Al did not cause
2 any obvious side effect on body weight of mice (**Figure 8a**). On day 18 post tumor challenge,
3 photographic images of lungs from immunized mice revealed that TCL@EGCG/Al
4 formulations remarkably decreased the number of tumor nodules in the lung compared to PBS
5 and TCL groups (Figure 8b and **Figure S6**). The result indicated that the vaccination of
6 TCL@EGCG/Al prevented the establishment of pulmonary tumor nodules. Besides,
7 TCL@EGCG/Al exhibited the comparative antitumor effect with TCL+PIC in B16 pulmonary
8 metastasis model. HE staining of lung sections indicated the significant histological changes of
9 the lungs in PBS and TCL groups due to the tumor nodules (Figure 8c). The enlarged
10 photographic images of lung were analyzed by Image J (Figure 8d). And the result illustrated
11 that TCL@EGCG/Al and TCL+PIC showed a similar trend in the expansion of tumor area in
12 lung. Furthermore, TCL@EGCG/Al significantly reduced the expansion of tumor area in lung
13 compared to PBS and TCL groups.

14 The total serum IgG levels of mice after different treatments were also tested (Figure 8e).
15 There was no statistically significant difference between the four groups with respect to total
16 IgG levels, suggesting that the antitumor effect of TCL@EGCG/Al was not caused by antibody
17 immunity. Furthermore, the cytokine levels (TNF- α , IL-10 and IL-4) of splenocyte culture
18 supernatant after 3 days of antigen restimulation were performed. Splenocyte from
19 TCL@EGCG/Al-treated mice produced greater level of TNF- α , which are associated with
20 enhanced protection than mice treated with other formulations (Figure 8f). Simultaneously,
21 TCL@EGCG/Al did not increase the immunosuppressive cytokine IL-10 (Figure 8g) and the
22 Th2-associated cytokine IL-4 (**Figure S7**), indicating that TCL@EGCG/Al did not increase the
23 proportions of IL-4/IL-10-producing negative regulatory CD4⁺ T cells. It was all known that
24 negative regulatory CD4⁺ T cells were detrimental to tumor immunity. In summary, the
25 obtained TCL@EGCG/Al microparticles has superior antitumor effect in B16 pulmonary
26 metastasis in vivo.



1
 2 **Figure 8.** (a) Growth curves of body weight of mice after various treatments, the data are
 3 presented as mean \pm SEM (n=6), (b) Photographic images of lungs from tumor-bearing mice
 4 on day 18 post tumor challenge, (c) Hematoxylin and eosin (HE) staining of mice lung sections
 5 after different treatments, the magnifications of all images were 4 \times , (d) Analysis of the enlarged
 6 photographic images of lung in Figure S6 by Image J, and the data were given by the area of

1 tumor divided by the area of entire lung (Area%), the data are presented as mean \pm SEM (n=6)
2 (e) The total serum IgG levels of mice on day 18, (f-g) TNF- α and IL-10 levels of splenocyte
3 culture supernatant after 3 days of antigen stimulation, data are expressed as mean \pm SD (n =
4 6). The differences were analyzed by one way ANOVA with Bonferroni multiple comparison
5 post-test, ***p < 0.001.

6 **3. Conclusion**

7 A generic and effective approach to encapsulation of individual cells has been developed by
8 the formation of EGCG/Al coating onto tumor cells. The coating process can be rapidly
9 completed under mild condition, and the materials involved in our strategy are generally
10 regarded as safe by the US Food and Drug Administration. Almost all of the proteins in tumor
11 cell was entrapped within the microparticles, avoiding the antigen loss. Moreover, the
12 coordination bond endowed the microparticles with pH-responsive protein release. More
13 importantly, the microparticles enhanced the uptake efficiency of antigens, protected antigens
14 from degradation in vivo, and delayed the retention time of antigens in lymph node. In addition,
15 the microparticles has the comparative antitumor effect with PolyI:C in pulmonary metastasis
16 model. In principle, this simple and flexible strategy could be extended to other tumor cell types,
17 and provides a novel avenue to personalized cancer immunotherapy.

18 **4. Experimental Section**

19 *Materials:* 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), aluminium
20 chloride hexahydrate (AlCl₃·6H₂O) and fluorescein isothiocyanate (FITC) were purchased
21 from Sigma-Aldrich. Epigallocatechin-3-gallate (EGCG, 95%) was purchased from Melone
22 Pharmaceutical Co. Ltd (China). RPMI-1640, DMEM, and fetal bovine serum (FBS) were
23 purchased from Thermofisher (USA). DAPI, red blood cell lysis and cell counting kit-8 (CCK-
24 8) were purchased from Solarbio Science & Technology Co. Ltd (China). Cy7 NHS ester was
25 purchased from ApexBio (USA). BCA Protein Assay Kit and Dil were purchased from
26 Beyotime (China). Poly(I:C) (PIC, HMW VacGrade™, catalog# vac-pic) were purchased

1 from InvivoGen (USA). Recombinant mouse GM-CSF and IL-4 were purchased from
2 Peprotech (Rocky, Hill, USA). Anti-mouse monoclonal antibodies (CD80 (B7-1)-FITC, MHC I
3 (H-2Kb)-APC, MHC II (I-Ab)-FITC, CD11c-PerCP-Cy5.5 and CD40-PE) were purchased
4 from eBioscience (USA). Mouse Total IgG ELISA Kit was purchased from neobioscience
5 (China). Mouse cytokines ELISA kit (IL-10, TNF- α , IL-4) were purchased from ebioscience
6 (USA). All chemicals were used without further purification.

7 *Cell lines and animals:* The immortalized mouse dendritic cells line DC2.4, murine melanoma
8 cells line B16 and human acute myeloid leukemia cells line HL-60 were purchased from the
9 Cell Bank of China Academy of Sciences, and cultured according to the manufacture's
10 guidelines. Mouse urothelial carcinoma cells line MB49, colon carcinoma cell line CT26, breast
11 cancer cell line 4T1 were purchased from China Infrastructure of Cell Line Resource. Female
12 C57BL/6 (6-8 weeks old) mice were purchased from Vital River Laboratory Animal
13 Technology Co., Ltd. (China), and raised under SPF conditions. All animals operation was in
14 compliance with the regulations of the Tianjin Committee of Use and Care of Laboratory
15 Animals, and the overall project protocol was approved by the Animal Ethics Committee of the
16 Chinese Academy of Medical Science.

17 *Encapsulation of tumor cells and preparation of TCL@EGCG/Al:* B16 cells were detached and
18 single cell suspension was obtained (2×10^6 cells/mL). Then, EGCG (0.5 mL, 20 mg mL⁻¹) and
19 AlCl₃ (0.5 mL, 5 mg mL⁻¹) aqueous solutions were added to 9 mL of the cell suspension. The
20 cell suspension was vigorously mixed for 60 s immediately after the additions of EGCG and
21 AlCl₃. Then the cells were centrifuged (200 g, 3 min) and washed with isotonic saline solution
22 to remove excess EGCG and AlCl₃ after each layer deposition. This coating process was
23 repeated three times, B16@EGCG/Al were prepared. After coating, the cells were incubated in
24 high-purity water for 30 min to kill the cells, and tumor cell lysates (TCL)-containing
25 microparticles (TCL@EGCG/Al) were obtained. The whole process was performed at 4 °C to
26 block energy-dependent internalization pathways. The loading capacity represented the ratio of

1 protein amount encapsulated in the microparticles to the microparticles weight. SEM and TEM
2 images of TCL@EGCG/Al were performed. As a control, TCL was obtained by five repeated
3 freeze-thaw cycles as previously reported in the literature, and diluted to required concentration
4 before use.^[35]

5 4T1, CT26, MB49 and HL-60 cells were also encapsulated by EGCG/Al layer, then CLSM
6 images and SEM images of these cells before and after coating were conducted. Uncoated cells
7 were co-cultured with FITC-containing DMSO and medium mixture at 37 °C for 30 min, then
8 the FITC-labelled cells were washed twice with PBS. CLSM images of uncoated cells were
9 conducted after fixation of FITC-labelled cells with paraformaldehyde followed by labeled with
10 DAPI. CLSM images of the coated cells were also conducted after the coating of FITC-labelled
11 cells followed by labeled with DAPI. TEM images of uncoated cells were fixed by
12 glutaraldehyde followed by washing with high-purity water.

13 *Proteins release from TCL@EGCG/Al:* The amount of proteins released from TCL@EGCG/Al
14 were measured under pH 5.5 and pH 7.4. TCL@EGCG/Al containing 4 mg of TCL were
15 suspended in 4 mL of PBS and incubated at 37 °C with continuous agitation in an orbital shaker.
16 The suspension was centrifuged at 3000 rpm for 5 min at designated time points. 2 mL of
17 supernatants were collected and stored at -80 °C for analysis. Then 2 mL of fresh PBS was
18 added to the original tube. Proteins in supernatants were tested using BCA assay. The
19 cumulative release of protein was presented as a function of time.

20 *Stability of TCL@EGCG/Al:* The stability of TCL@EGCG/Al was evaluated by suspending the
21 microparticles vaccine in culture medium containing a known concentration of BSA or FBS
22 and incubated at 37 °C. At regular intervals, the microparticles suspension was centrifuged and
23 the concentration of BSA or FBS in the supernatant was measured by BCA. Then, the
24 concentration of BSA or FBS was given as a function of time. Besides, optical images were

1 taken after suspending the microparticles in culture medium containing BSA or FBS for a
2 period of time.

3 *Generation of BMDCs and antigens uptake:* C57BL/6 mice were sacrificed and bone marrow
4 was flushed out of femur and tibia. After red blood cell lysis, cells were seeded in six-well
5 plates (2×10^5 cells/ml) and incubated at 37 °C in 5% CO₂. Culture medium was RPMI-1640
6 supplemented with 10% FBS, 1% penicillin/streptomycin, 10 ng/ml IL-4 and 20 ng/ml GM-
7 CSF. On day 6 of culture, the non-adherent cells were harvested and immature BMDC cells
8 were obtained. B16 cells were labeled with FITC according to the reported method,^[25, 36] then,
9 TCL-FITC and TCL-FITC@EGCG/Al were obtained with FITC-labeled B16 cells according
10 to the preparation process of TCL and TCL@EGCG/Al. And the fluorescent spectra of TCL-
11 FITC and TCL-FITC@EGCG/Al were presented in **Figure S1**. Immature BMDCs (5×10^5
12 cells/mL) were cultured with TCL-FITC@EGCG/Al for 8 h. After the incubation, the cells
13 were washed twice with PBS, stained with DAPI and Dil. The fluorescent images were recorded
14 by CLSM (CarlZeiss LSM710). DC2.4 cells were seeded in 24-well plate (1×10^6 cells/well)
15 and incubated with TCL-FITC and TCL-FITC@EGCG/Al for 6 h, the dosage of TCL-FITC
16 was 20 ug/well. After extensive washing, the antigen uptake was determined by FACS analysis.

17 *Stimulation and activation of BMDCs:* Immature BMDCs were plated in 24-well plates (5×10^5
18 cells/ml). Then, TCL and TCL@EGCG/Al were added to make final TCL concentration of 10
19 µg/ml. After 48 h of culture, cells were centrifuged and analyzed by FACS analysis (BD
20 Pharmingen) after staining with CD11c in combination with CD40 and CD80 or MHCI and
21 MHCII. And the supernatant was collected and stored at -80 °C for cytokines determination by
22 using a mouse Magnetic Luminex Screening assay for IL-12p70, TNF-α, IFN-γ, IL-6, IL-4 and
23 IL-10 according to the manufacturer's protocol (R&D Systems) on a Luminex 200. Untreated
24 cells and cells incubated with LPS served as negative and positive controls, respectively.

25 *In vivo trafficking of TCL@EGCG/Al:* To prepare TCL-Cy7 and TCL-Cy7@EGCG/Al, B16
26 cells were first labeled with Cy7 according to the labeling method of FITC. Then, TCL-Cy7

1 and TCL-Cy7@EGCG/Al were obtained with Cy7-labeled B16 according to the preparation
2 process of TCL and TCL@EGCG/Al as described above. And the fluorescent spectra of TCL-
3 Cy7 and TCL-Cy7@EGCG/Al were presented in **Figure S2**. Female C57BL/6 mice were
4 injected subcutaneously at the tail base with TCL-Cy7 and TCL-Cy7@EGCG/Al containing
5 150 µg of TCL-Cy7 dispersed in 100 µl PBS. Then mice were visualized at the injection site
6 and the adjacent lymph node (LN), where immune responses were initiated.^[37] Vaccine kinetics
7 was then studied at several time points after administration by *in vivo* imaging system (Maestro,
8 CRI USA). Mice were anesthetized by inhalation of isoflurane and fluorescence spectral cubes
9 were acquired using near infrared preset filter. Unmixed images in which background signals
10 were subtracted and quantified by using Maestro software.

11 *Antitumor therapeutic of TCL@EGCG/Al in B16 pulmonary metastasis model:* Female 6-8
12 weeks C57BL/6 mice were i.v. injected via tail vein with 1×10^5 B16 cells suspended in 100 µL
13 PBS at day 0, and randomly assigned into four groups (n=6): 1) Control, 2) TCL, 3) TCL+PIC,
14 and 4) TCL@EGCG/Al. Mice were monitored every other day for body weight. On day 4, 9
15 and 14, mice were s.c. immunized with 100 µL the corresponding samples containing 200 µg
16 of TCL. Mice were sacrificed at day 18 by cervical dislocation, and the spleen and lung were
17 isolated for further analysis. Venous blood of mice was collected and serum was isolated. Serum
18 total IgG level was measured using Mouse Total IgG ELISA kit. Lungs were fixed in 4%
19 formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE). Single
20 cells suspension of spleen was prepared and plated in 96-well plate (5×10^6 cells/ml),
21 restimulated with 40 µg mL^{-1} of TCL for 72 h, then the culture supernatant was collected for
22 determining cytokine concentrations by ELISA assay.

23 *Statistical Analysis:* All results except that in Figure 8a,d, which are presented as the mean ±
24 standard error of the mean (SEM), are presented as the mean ± standard deviation (SD), as
25 indicated. Statistical analysis for all tests except that in Figure 6b, which is carried out using
26 unpaired Student's t-test, is carried out using one way ANOVA with Bonferroni's Multiple

1 Comparison Test. Sample size (n) for all statistical analysis except that in Figure 4c-e and
 2 Figure 8 (n=6) is 3. All statistics were performed in GraphPad Prism (PRISM7.0; GraphPad
 3 Software). The threshold for statistical significance was $P < 0.05$.

4 **Supporting Information**

5 Supporting Information S1 and Supporting Information Video S2 are available from the Wiley
 6 Online Library or from the author.

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2

3 Supporting Information

4

5

6 **A generic coordination assembly-enabled nanocoating of**
7 **individual tumor cells for personalized immunotherapy**

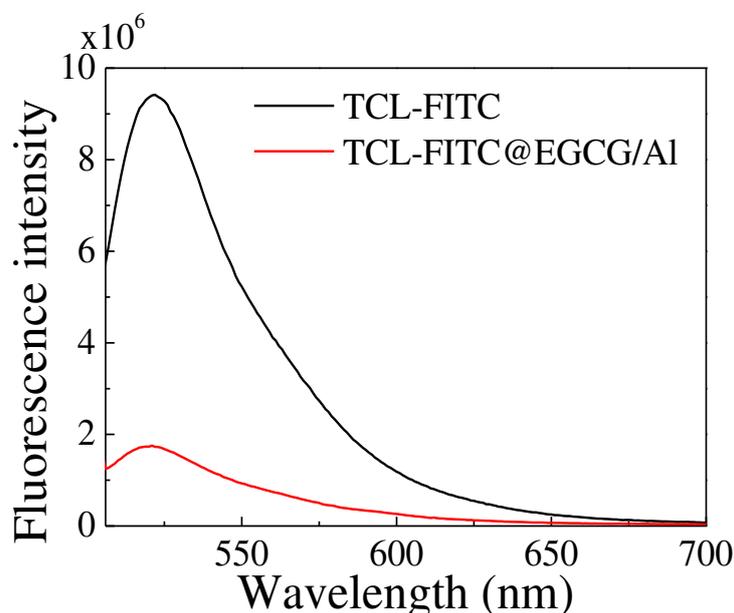
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9 *Xiaoli Wang,^{‡a} Zuoguan Chen,^{‡b} Chao Zhang,^a Chuangnian Zhang,^a Guilei Ma,^{*a} Jing*
10 *Yang,^a Xiaoqing Wei,^{*c} Hongfan Sun^a*

11

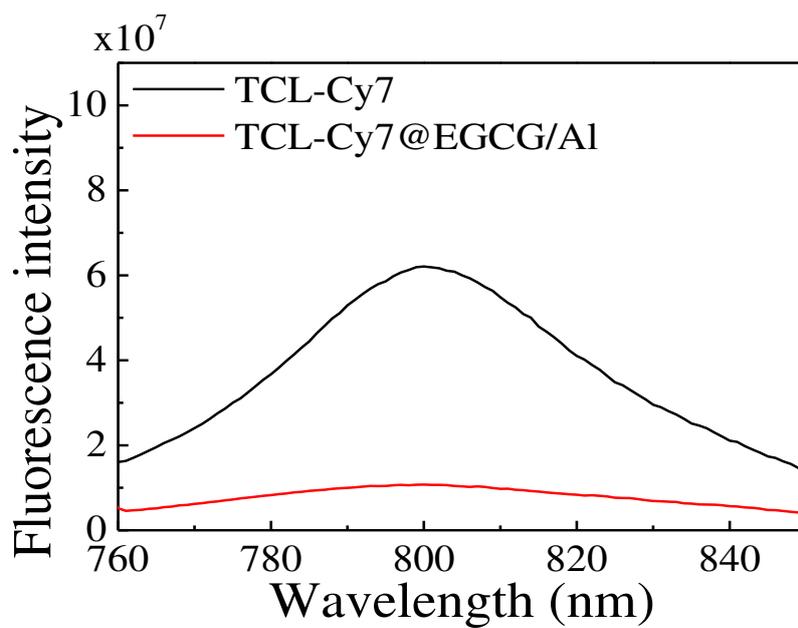
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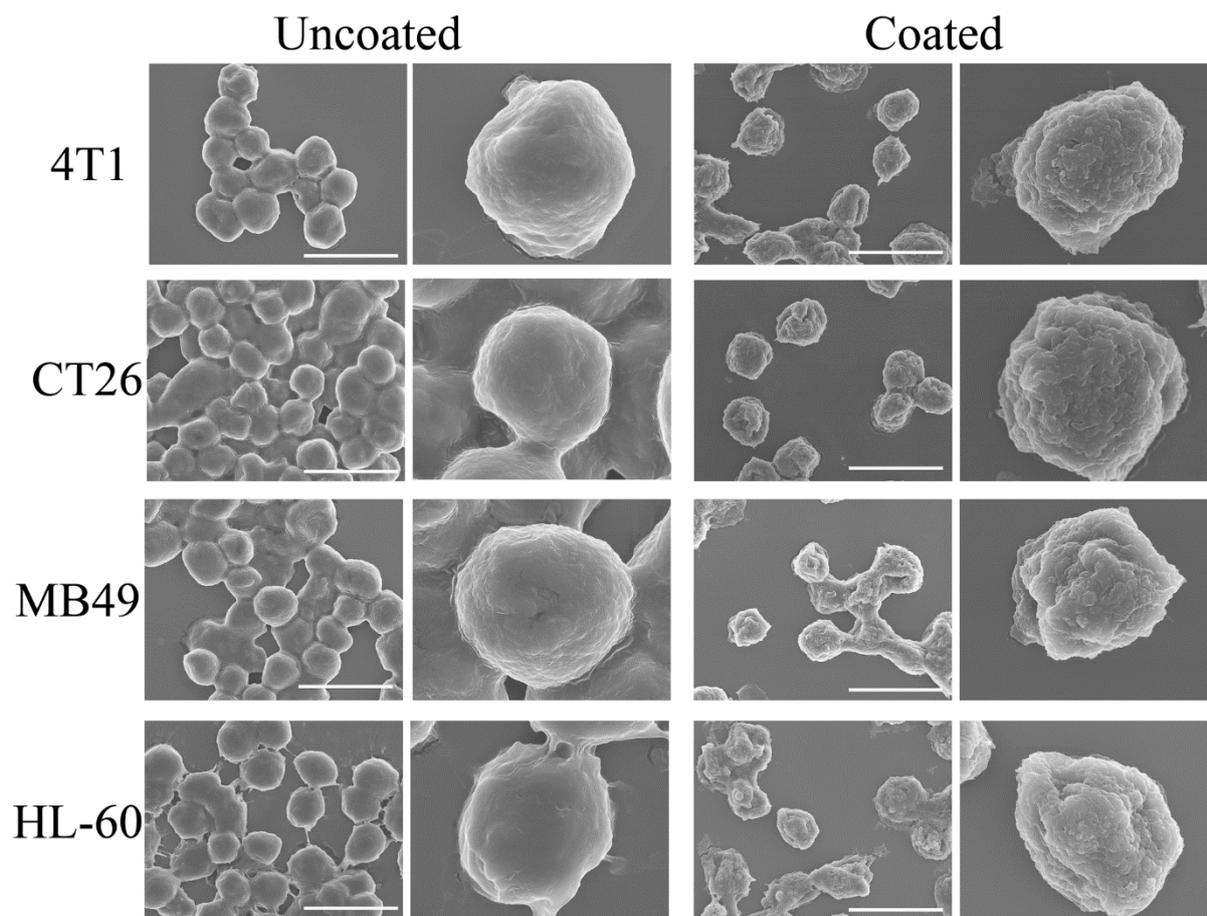
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15 **Figure S1.** The fluorescence spectra of TCL-FITC and TCL-FITC@EGCG/Al in water, the
16 excitation wavelength was 495 nm, the concentration of TCL-FITC in all the formulations was
17 10 $\mu\text{g ml}^{-1}$. The fluorescence intensity of TCL-FITC was obviously higher than that of TCL-
18 FITC@EGCG/Al although the concentration of TCL-FITC in all the formulations was the same.
19 This phenomenon was notoriously known as fluorescence aggregation-caused quenching
20 (ACQ).[1, 2] Because of the high protein loading capacities, the concentration of TCL-FITC
21 within microparticles were quite high, thus the aggregation of TCL-FITC caused fluorescence
22 quenching.



1
2 **Figure S2.** The fluorescence spectra of TCL-Cy7 and TCL-Cy7@EGCG/Al in water, the
3 excitation wavelength was 750 nm, the concentration of TCL-Cy7 in all the formulations was
4 0.3 mg ml^{-1} .

5



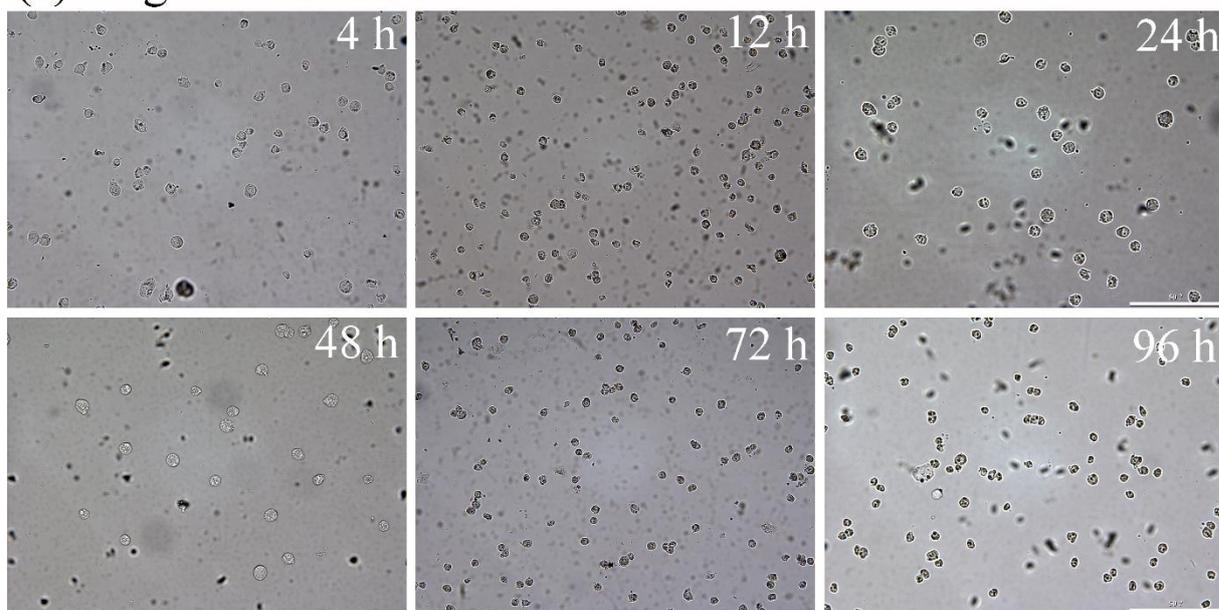
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2 **Figure S3.** SEM images of 4T1, CT26, MB49 and HL-60 cells before and after coating by
3 EGCG/Al layer. Uncoated cells were fixed by glutaraldehyde followed by washing with high-
4 purity water. The ruler was 20 μm .

5

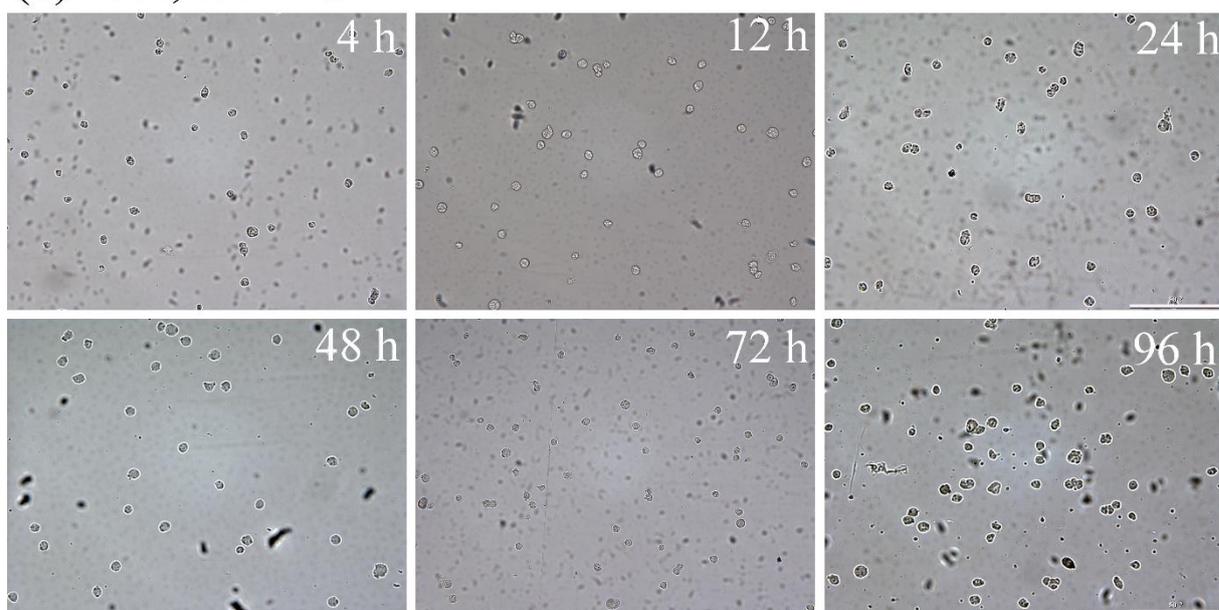
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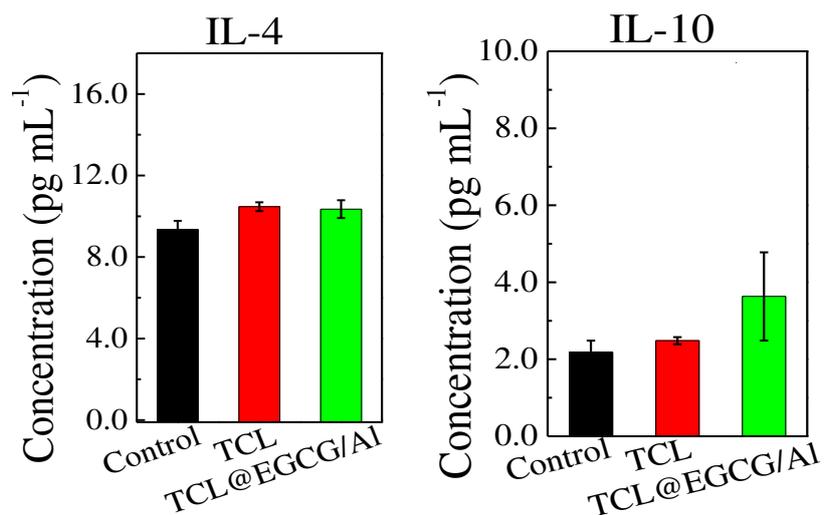
(a) 1mg mL⁻¹ of BSA



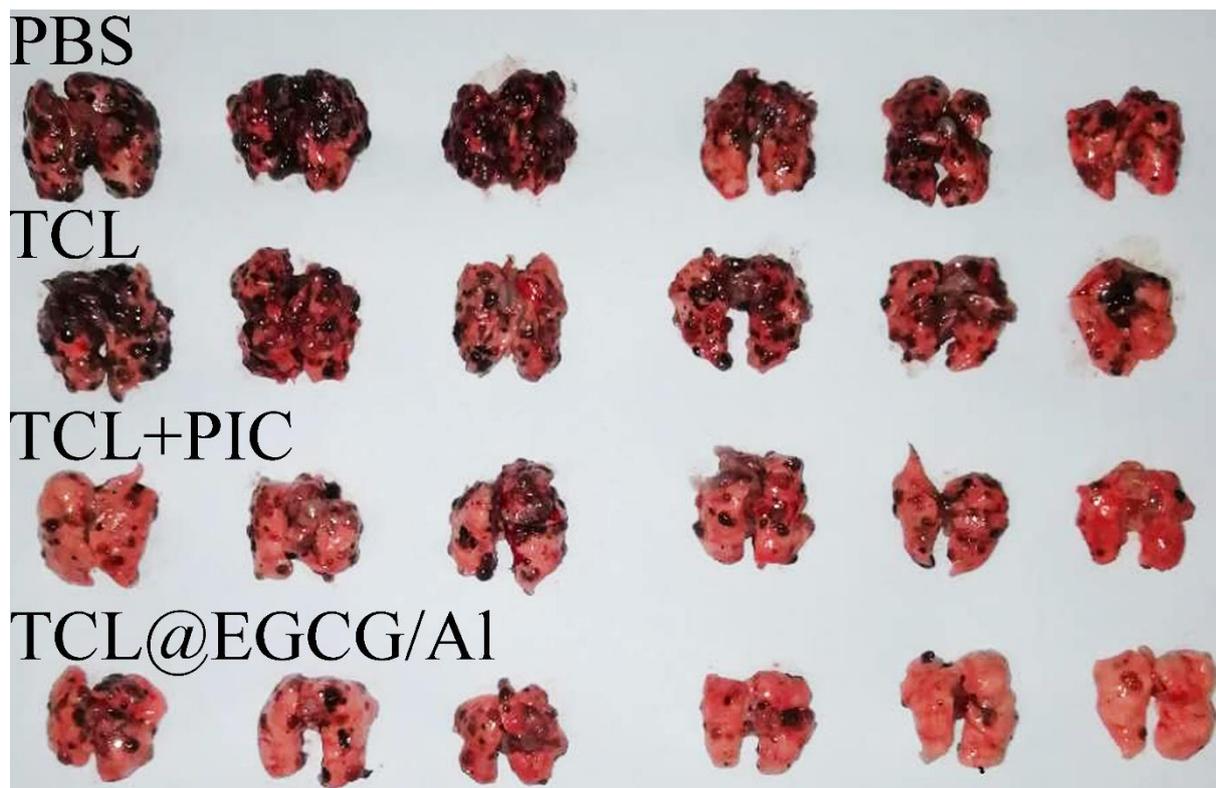
(b) 10%, v/v FBS



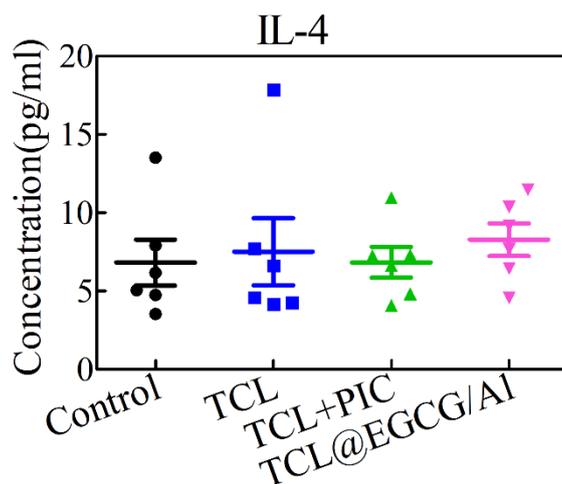
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2 **Figure S4.** The optical images of TCL@EGCG/Al after suspending the microparticles
3 vaccine in culture medium containing a known concentration of BSA or FBS at 37 °C for a
4 period of time.



1
2 **Figure S5.** Secretion of IL-4 and IL-10 from BMDCs treated with different formulations, the
3 differences were analyzed using unpaired student's t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
4



1
2 **Figure. S6** Photographic images of lungs from tumor-bearing mice on day 18 post tumor
3 challenge



8
9 **Figure. S7** IL-4 level of splenocyte culture supernatant after 3 days of antigen stimulation, the
10 data are expressed as mean \pm SD (n = 6), the differences were analyzed by one way ANOVA
11 with Bonferroni multiple comparison post-test.

12

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